Angiotensin-Converting Enzyme and Angiotensin II Receptor Subtype 1 Inhibitors Restitute Hypertensive Internal Anal Sphincter in the Spontaneously Hypertensive Rats

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ABSTRACT

The present study determined the effects of the angiotensin-converting enzyme (ACE) inhibitor captopril and angiotensin II receptor subtype 1 (AT$_1$-R) antagonist losartan on the internal anal sphincter pressures (IASP) in spontaneously hypertensive rats (SHR) versus normotensive Wistar-Kyoto rats (WKY). The SHR had significantly higher IASP (21.7 ± 0.8 mm Hg) than the WKY (14.7 ± 0.9 mm Hg), which was associated with the higher levels of angiotensin II (Ang II) in plasma (50.3 ± 8.5 pg/ml) and in muscle bath perfusates (72.7 ± 11.8 pg/ml) compared with the WKY (p < 0.05). Captopril and losartan decreased the IASP in SHR and WKY, but they were more potent in SHR. Captopril and losartan normalized the IASP in the SHR, whereas these agents may compromise rectoanal continence in the WKY. Reverse transcriptase-polymerase chain reaction and Western blots showed higher levels of angiotensinogen, renin, ACE, and AT$_1$-R in the internal anal sphincter (IAS) of SHR. Ang II caused concentration-dependent contraction of IAS smooth muscle strips from WKY (pEC$_{50}$ = 8.5 ± 0.1) and SHR (pEC$_{50}$ = 8.6 ± 0.2). Losartan (100 nM) significantly (p < 0.05) inhibited this effect. From these data, we conclude that 1) hypertensive IAS is primarily the result of renin-angiotensin system up-regulation, 2) ACE inhibitors and AT$_1$-R antagonists simply relieve the hypertensive IAS, and 3) the differential effect of these inhibitors in the hypertensive versus the normotensive IAS may explain the lack of incontinence as a side effect in hypertensive patients receiving ACE inhibitors and AT$_1$-R antagonists.

Earlier studies from our laboratory have put forward evidence for the role of angiotensin II (Ang II) biosynthesis in the spontaneous myogenic tone of the internal anal sphincter (IAS) of rats (De Godoy et al., 2004). The studies show the expression of key components of the renin-angiotensin system (RAS): angiotensinogen (Angen), the Ang I-generating enzyme renin, and the Ang I-converting enzyme (ACE). Additional studies suggest an autocrine mechanism for Ang II generation in the regulation of the IAS basal tone (De Godoy et al., 2004; De Godoy and Rattan, 2005). These findings are in line with a broad range of reports in different tissues (Dzau et al., 1987, 1988; Eggena et al., 1990; Griending et al., 1993; Katwa et al., 1996; Bataller et al., 2003). These data expand the role of the RAS from endocrine to local regulation.

The role of Ang II in different organ systems can be determined by the classic ACE inhibitors or by the Ang II type 1 receptor (AT$_1$-R) antagonists, captopril and losartan, respectively. Captopril was first described in 1978 as a chronic antihypertensive agent in hypertensive rats by inhibiting the conversion of Ang I into Ang II by ACE (Rubin et al., 1978). Losartan was introduced later as an alternative to captopril for the treatment of hypertension (Celik et al., 1995).

In previous in vitro studies we showed that inhibition of Ang II generation decreases IAS basal tone (De Godoy et al., 2004; De Godoy and Rattan, 2005), suggesting the potential use of Ang II inhibitors in certain gastrointestinal disorders characterized by the hypertensive IAS. However, a direct role of the RAS and Ang II in hypertensive IAS pressure (IASP) has not been examined.
The goal of the present studies was to evaluate the role of RAS, and the effects of Ang II inhibitors in the IASP of normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). We carried out in vivo and in vitro, functional, biochemical, and molecular biology experiments in these animal models. We also examined the effects of captopril and losartan in the IASP. We systematically compared these parameters in the tonic smooth muscles of the IAS versus those of the rectum (RSM), and the aorta (AoSM). The RSM and AoSM represent phenotypes of phasic smooth muscles.

Materials and Methods

Measurements of Intraluminal Pressures in the IASP. WKY and SHR were anesthetized with isoflurane (initially with 5% isoflurane and then maintained with 1% isoflurane throughout the length of the experiment). The IASP was measured via a solid-state transducer/catheter model 2-Fr Mikro-Tip (Millar Instruments, Houston, TX), following the previously described method of Terauchi et al. (2005). The catheter assembly was initially introduced into the rectum and then positioned precisely in the IAS via slow-pull-through (approximately 7–8 mm from the anal verge). High pressures of the IAS consisted of rhythmic fluctuations superimposed on the steady tone. The IASP was recorded using a PowerLab/8SP recorder and analyzed via the software Chart 4 PowerLab (ADInstruments, Inc., Colorado Springs, CO).

Systolic Blood Pressure Measurements. The systolic blood pressure (SBP) was recorded via a catheter (i.d. 0.38 mm; Clay Adams, Parsippany, NJ) attached to a Statham transducer (Medex, Inc., Carlslad, CA). All recordings and analyses were carried out using Chart 4 PowerLab (ADInstruments, Inc.).

Administration of Agents. IASP and SBP were monitored for 30 min after the additive administration of captopril and losartan (1–50 mg/kg each, over 1 min of infusion). These doses have been shown to effectively reduce isometric tension in these animal models. We also examined the effects of captopril and losartan in the IASP. We systematically compared these parameters in the tonic smooth muscles of the IAS versus those of the rectum (RSM), and the aorta (AoSM). The RSM and AoSM represent phenotypes of phasic smooth muscles.

Cumulative concentration-response curves for Ang II (0.1 nM–10 μM) in the IAS were obtained before and after 100 nM losartan, both in the background of 10 μM captopril. The changes in IAS basal tone were expressed as the percentage of maximal contraction by 10 μM bethanecol. The losartan concentration used in this study was previously shown to be effective (Fan et al., 2002; De Godoy et al., 2004). The experimental protocols for in vivo and in vitro studies were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University and were in accordance with the recommendations of the American Association for the Accreditation of Laboratory Animal Care.

Ang II Determinations. Arterial blood (2 ml) was rapidly collected into a chilled (4°C) tube containing 1 mg/ml EDTA and 1 μM amastatin (inhibitor of the enzymes that degrade Ang II) (De Godoy et al., 2004). The blood was centrifuged at 4000 g for 10 min, and the plasma (1 ml) was immediately applied to an octadecasilicyl (C₈)-silica cartridge (Waters Corp., Milford, MA). Samples were also collected as 2 ml of the muscle bath perfusates (MBPs) after incubation of the IAS, RSM, and AoSM tissues for 30 min in the presence of 1 μM amastatin and immediately applied to C₈. All samples were washed with 10 ml of 0.1% trifuluoroacetic acid. The peptides were eluted with 6 ml of methanol-water-trifuluoroacetic acid (80:19:1, v/v/v). The eluate was dried in a vacuum centrifuge and stored at 4°C until the further procedures (Bagby et al., 1979; Aihara et al., 1999). The eluate was dissolved in 300 μl of PBS, transferred onto a 96-well React-Bind NeutrAvidin-coated enzyme-linked immunosorbent assay plate (Pierce, Rockford, IL), and incubated for 1 h at room temperature. Nonspecific binding was blocked with blocking buffer (PBS, 0.1% bovine serum albumin, and 0.05% Tween 20) for 30 min at room temperature. Then, each well was washed 3 times with washing buffer (PBS and 0.05% Tween 20) and incubated for 60 min at room temperature with 100 μl of rabbit anti-Ang II antibody diluted in blocking buffer. Wells were washed and incubated for 60 min at room temperature with the donkey adsorbed anti-rabbit secondary antibody in blocking buffer. Wells were washed and exposed to the 1-Step TMB Substrate (Pierce) for 30 min at room temperature. Finally, the reaction was stopped by 2 M H₂SO₄, and the plate was read at 450 nm in an enzyme-linked immunosorbent assay plate reader. Our preliminary experiments determined optimal conditions to be 1:1000 and 1:2000 for primary and secondary antibodies (De Godoy and Rattan, 2005). The levels of Ang II in plasma and MBP were determined in the basal state and in the presence of captopril or losartan (50 mg/kg and 10 μM each in the case of plasma and MBP, respectively).

RT-PCR. Total RNA was isolated and purified from different tissues by the acid guanidine-phenol-chloroform method (Chomczynski and Sacchi, 1987) and quantified by measurement of absorbance at 280 nm in a spectrophotometer. Total RNA (2 μg) was subjected to first-strand cDNA synthesis using oligo(dT) primers (Promega, Madison, WI) and an Omniscript RT Kit (Qiagen, Germantown, MD) in a final volume of 20 μl at 42°C for 60 min. PCR primers specific for Angen, renin, ACE, AT₁-R, and α-actin cDNA were designed and are shown in Table 1. PCR was performed in a Promega 2× Master Mix

<table>
<thead>
<tr>
<th>Primer</th>
<th>Strand</th>
<th>Sequence (5′→3′)</th>
<th>Accession No.</th>
</tr>
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<tr>
<td>ACE</td>
<td>Reverse</td>
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<td>NM_019544</td>
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<td>Renin</td>
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<tr>
<td>AT₁-R</td>
<td>Reverse</td>
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<td>NM_031009</td>
</tr>
<tr>
<td>α-Actin</td>
<td>Forward</td>
<td>GCCACAAACCCGGGAAAGG</td>
<td>NM_007392</td>
</tr>
</tbody>
</table>

TABLE 1
Primers used in the PCR for amplification of cDNA encoding angiotensinogen, ACE, renin, and α-actin in the IAS, RSM, and AoSM of WKY and SHR
Promega) in a final volume of 25 µl, using a PerkinElmer Thermal Cycler (PerkinElmer Life and Analytical Sciences, Boston, MA). The PCR conditions (in the case of Angen, renin, and α-actin) consisted of 94°C for 5 min (for the initial denaturation phase) followed by 35 cycles of 94°C for 30 s (denaturation), 57°C for 30 s (annealing), and 72°C for 1 min (extension). In the end, a final extension at 72°C for 7 min was allowed. In the case of ACE and AT1-R, the annealing temperature was set at 60°C. The PCR products were separated on 1.5% (w/v) agarose gel containing ethidium bromide and were visualized with UV light. The relative densities of Angen, renin, ACE, and AT1-R were calculated by normalizing the integrated optical density (IOD) of each blot with that of α-actin.

Western Blot Analysis. Western blot studies were performed to determine the relative distribution of Angen, renin, ACE, and AT1-R following the previous method (Fan et al., 2002). In brief, the tissues isolated and dissected as described above were subjected to homogenization, protein extraction, and determination by the method of Lowry et al. (1951). The proteins (20 µg) were then separated by gel electrophoresis and transferred onto a nitrocellulose membrane (NCM) at 4°C.

The NCM was then incubated with the specific primary antibodies (mouse IgG, 1:1000 for Angen and renin; goat IgG, 1:1000 for ACE; and rabbit IgG 1:1000 for AT1-R) for 2 h at room temperature. After washing with Tris-buffered solution-Tween 20, the NCMs were incubated with horseradish peroxidase-labeled secondary antibody (1:10,000) for 1 h at room temperature. The corresponding bands were visualized with enhanced chemiluminescence substrate using the SuperSignal West Pico Chemiluminescent Substrate (Pierce) and Hyperfilm MP (Amerham Biosciences Inc., Piscataway, NJ).

NCMs were then stripped of antibodies using the Restore Western Blot Stripping Buffer (Pierce) for 5 min at room temperature and reprobed for α-actin using the specific primary (mouse IgG 1:10,000 for α-actin) and secondary (1:10,000) antibodies. Bands corresponding to different proteins were scanned (SnapSanc.310; Agfa, Ridgefield Park, NJ), and the IODs were determined using Image-Pro Plus.

![Fig. 1. A and B, comparison of the basal levels of (A) IASP and (B) SBP between WKY and the SHR reveals higher levels in the SHR group (+, p < 0.05; unpaired Student’s t test; n = 6–7). Data in this and the subsequent figures represent means ± S.E.M.](image-url)

![Fig. 2. Effects of different doses of the ACE inhibitor captopril on the IASP of WKY and SHR (A) and the respective representative tracings (B and C). Captopril is more potent in causing a fall in the IASP of SHR versus WKY (+, p < 0.05; one-way ANOVA; n = 4).](image-url)
Captopril and losartan was a generous gift from Merck (Rahway, NJ). Ang II antibody was from Peninsula Laboratories, Inc. (San Carlos, CA). Angen antibody was from Research Diagnosis, Inc. (Flanders, NJ). All other antibodies were from Santa Cruz Biochemicals (Santa Cruz, CA). All PCR primers were from MWG Biotech (High Point, NC).

**Data Analysis.** Results were expressed as means ± S.E.M. Concentration-response/dose-response curves were analyzed using a nonlinear interactive fitting program (Prism 3.0; GraphPad Software Inc., San Diego, CA). Agonist potencies and maximal responses were expressed as pEC50 (negative logarithm of the molar concentration of agonist producing 50% of the maximal response) and I_max (maximal inhibition elicited by the inhibitor, respectively). Inhibitor potencies and maximal inhibition were expressed as log ID50 (logarithm of the milligram per kilogram dose of inhibitor producing 50% of the maximal inhibition) and I_max (maximal inhibition elicited by the inhibitor), respectively. Statistical significance was tested by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test when three or more different groups were compared. To compare only two different groups, the unpaired Student’s t test was used. A p value < 0.05 was considered to be statistically significant.

**Results**

**Comparisons of the IASP, and SBP in WKY versus SHR.** The IASP in the SHR group was significantly higher than that in the WKY group with the values of 21.7 ± 0.8 and 14.7 ± 0.9 mm Hg, respectively (*, p < 0.05; n = 6–7) (Fig. 1A). SBP was also significantly (p < 0.05) higher in the SHR than in the WKY group with the values of 172.2 ± 1.9 and 115.5 ± 2.8 mm Hg, respectively (*, p < 0.05; n = 6) (Fig. 1B).

**Effects of Captopril and Losartan on the IASP.** Captopril and losartan significantly (p < 0.05) reduced the IASP in both strains of rats (Figs. 2 and 3). However, these agents were more efficacious and potent in the SHR (Table 2). For example, 5 mg/kg captopril produced a significantly greater fall in the IAS in the SHR versus WKY, 6.7 ± 0.8 and 1.5 ± 0.3 mm Hg, respectively (*, p < 0.05; n = 4) (Fig. 2). Interestingly, the final IASP for WKY after 50 mg/kg captopril was significantly lower (7.9 ± 0.4 mm Hg) (Fig. 4A) than that in the SHR (9.4 ± 0.4 mm Hg) (Fig. 4B). An actual defecation reflex was observed after captopril (50 mg/kg) in one of four WKY, in which the pressures from the IAS fell down to levels not significantly different from those of the rectum (3.5 ± 2.5 mm Hg). This suggests that higher doses of captopril may compromise IAS tone, one of the components of rectoanal continence.

Likewise, 5 mg/kg losartan produced a significantly greater fall in the IASP in SHR versus WKY, 7.0 ± 0.6 and 3.3 ± 0.3 mm Hg (*, p < 0.05; n = 4) (Fig. 3). It is noteworthy that in SHR although captopril and losartan were more potent in causing the fall in the IASP in absolute terms, these

<table>
<thead>
<tr>
<th>Group</th>
<th>log ID50</th>
<th>I max</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (captopril)</td>
<td>1.0 ± 0.1</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>WKY (losartan)</td>
<td>0.5 ± 0.13</td>
<td>6.3 ± 2.1</td>
</tr>
<tr>
<td>SHR (captopril)</td>
<td>0.42 ± 0.14</td>
<td>12.3 ± 2.3</td>
</tr>
<tr>
<td>SHR (losartan)</td>
<td>0.42 ± 0.2</td>
<td>10.0 ± 2.7</td>
</tr>
</tbody>
</table>

* log ID50 is defined as the logarithm of milligram per kilogram dose of the inhibitor that produces 50% inhibition. Data represent the mean ± S.E.M. of six to seven independent determinations.

* I max is defined as the maximal inhibition elicited by the inhibitor.

p < 0.05; one-way ANOVA; n = 4.

**4.0. The relative densities were calculated by normalizing the IOD of each blot with that of α-actin.**

**Drugs and Antibodies.** Ang II, amastatin, bethanechol, captopril, and HOE-140 were from Sigma-Aldrich (St. Louis, MO). Losartan was more potent in causing a fall in the IASP in SHR compared on the IASP of WKY (**, p < 0.05).**

**Fig. 3. Effects of different doses of the Ang II AT1-R antagonist losartan on the IASP of WKY and SHR (A) and their respective tracings (B and C). Losartan is more potent in causing a fall in the IASP in SHR compared with WKY (**, p < 0.05; one-way ANOVA; n = 4).**
agents simply normalized the IASP to the levels not significantly different from those in the WKY group (Table 2).

**Effects of Captopril and Losartan on SBP.** Captopril and losartan caused a significant fall in SBP of SHR but not WKY (Fig. 5). However, the final values for SHR, even after the higher doses of these agents, were not significantly different from those of WKY. The final SBPs after 50 mg/kg captopril and losartan for SHR were 122.2 ± 2.3 and 123.0 ± 6.3 mm Hg, respectively, and these values in the case of WKY rats before the administration of any of these agents were

114.3 ± 2.3 and 117.1 ± 5.7 mm Hg, respectively (p > 0.05; n = 4). Therefore, both the ACE inhibitor and AT1-R antagonist caused similar normalization of elevated SBP and IASP in the SHR.

**Comparison of Ang II Levels in Plasma of WKY and SHR.** Ang II levels in the plasma samples from SHR were significantly higher than those in the WKY (50.3 ± 0.9 pg/ml versus 13.2 ± 3.0 pg/ml, n = 3) (Fig. 6). Pretreatment with captopril (50 mg/kg) significantly reduced the circulating levels of Ang II in both groups of rats, confirming the efficacy of this agent as an ACE inhibitor.

Losartan (50 mg/kg) caused no significant change in the Ang II levels in the plasma samples from WKY, but caused a significant increase in SHR (*, p < 0.05) (Fig. 6). The higher levels of Ang II in the plasma may be explained on the bases of the displacement of Ang II from the binding sites from different tissues.

**Comparison of Basal IAS Tone and Ang II Levels in the MBPs from the IAS, RSM, and AoSM in WKY and SHR.** In agreement with the in vivo data, we observed a significant increase in the basal tone in the IAS from SHR versus WKY. The IAS tone in these groups of animals was 0.065 ± 0.013 and 0.025 ± 0.009 g/mg of the tissue weight, respectively (*, p < 0.05; n = 6) (Fig. 7A).

Ang II levels in the MBPs from the IAS (from both WKY and SHR) were higher than those from the RSM and AoSM (Fig. 7B). These differences were more dramatic between IAS and RSM from SHR, 72.7 ± 11.8 and 0.63 ± 0.19 pg/ml, respectively (*, p < 0.05; n = 3) (Fig. 7B).

Interestingly, in agreement with the data from the plasma samples, losartan pretreatment (10 μM) caused an increase in the levels of Ang II in the MBPs from the IAS tissues of SHR but not WKY, from 72.7 ± 11.8 to 115.7 ± 6.0 pg/ml (*, p < 0.05) (Fig. 7C). However, pretreatment of the IAS tissues with captopril (10 μM), as expected, caused a significant reduction in the levels of Ang II in both WKY and SHR.

**Western Blots to Determine the Relative Expression of Angen, Renin, ACE, and AT1-R in WKY and SHR.** Angen was observed as a doublet: 65- and 55-kDa bands (Fig. 8A). As previously demonstrated in rat pancreas (Leung et al., 1999) and in rat IAS (De Godoy and Rattan, 2005), the higher molecular weight band represents the proAngen, and the lower molecular weight band represents the renin substrate Angen. SHR samples expressed significantly (*, p < 0.05) higher levels of Angen (but not proAngen) than WKY samples (Fig. 8A). Expression of renin was also significantly higher in the IAS of SHR versus WKY (*, p < 0.05) (Fig. 8B), but not in the RSM and AoSM (p > 0.05).

ACE and AT1-R were found to be expressed in significantly higher levels in the IAS and AoSM of SHR versus WKY (+, p < 0.05) (Fig. 9, A and B). In the RSM of SHR, however, the levels of AT1-R were not significantly different from those of WKY.

**RT-PCR of Angen, Renin, ACE, and AT1-R in WKY and SHR.** To determine the role of the RAS in the elevated IASP and IAS tone in SHR versus WKY, we further evaluated the transcriptional patterns of the genes coding Angen, renin, ACE, and AT1-R. AoSM lacked RT-PCR products for renin (Fig. 10A). The expression patterns of AT1-R were significantly higher (Fig. 10D), lower in the case of Angen (Fig. 10A), and not significantly different for renin (Fig. 10B) and ACE (Fig. 10C). These differences in RT-PCR data (in contrast to the higher levels of Ang II, and translational expression of different components of the RAS in SHR versus WKY rats) suggest the regulation of RAS in Ang II generation to be at the post-transcriptional level.

**Contractile Effect of Ang II in the IAS.** Ang II in the presence of 10 μM captopril produced concentration-dependent contractions of the IAS smooth muscle strips from WKY and SHR, with similar E\(_{\text{max}}\) or pEC\(_{50}\) values (Table 3). Losartan (in the presence of captopril) produced a significant (p < 0.05) inhibition of the Ang II response. However, the smooth muscle strips from the SHR group were more resistant to losartan compared with those from the WKY group (p < 0.05) (Table 3; Fig. 11).
Discussion

The present studies demonstrate hypertensive IAS in SHR and its restitution to normotensive levels after the administration of ACE and AT₁-R inhibitors. Using a multipronged approach of functional, molecular biology and Ang II bioassay measurements, we show that the hypertensive IAS in these animals is associated with the up-regulation of the RAS.

There is an abundance of literature to show the relationship between the up-regulation of the RAS and the development of cardiovascular (CV) hypertension (Bagby et al., 1979; Hubner et al., 1995; Bolterman et al., 2005) in the SHR. In all of these studies normotensive WKY rats have been used as controls. Considering the recent concept of the role of the RAS in IAS tone (De Godoy and Rattan, 2005; Rattan, 2005), the present studies provide important information on the role of the RAS in the pathophysiology of IAS tone in the SHR. SHR develop significantly higher IASP (on anorectal manometry for the intraluminal pressures in in vivo studies) and IAS tone (in in vitro studies), suggesting a relationship between CV and IAS hypertension. A similar relationship has been reported in certain patients (Celik et al., 1995) with CV hypertension associated with hypertensive IAS and constipation. These symptoms disappeared after treatment with antihypertensive agents. The above changes in IAS tone in SHR appear to be selective because no such hyperactivity was observed in the RSM in these animals. Such data provide important future directions to explore the effects of ACE and AT₁-R inhibitors in motility disorders characterized by IAS hypertension.

Since their advent, captopril and losartan have been used extensively as antihypertensive agents because of their ability to inhibit Ang II generation and interaction with the AT₁-R, respectively (Rubin et al., 1978; Celik et al., 1995). The present data also demonstrate the normalization of the hypertensive IAS with both captopril (Fig. 2) and losartan (Fig. 3) in SHR on a par with the basal IASP in WKY. Similar results were also observed in parallel, showing normalization of SBP of SHR after these inhibitors (Fig. 5). Beyond normalization, these agents do not produce any hypotensive effect in

![Fig. 5. SBP data. Effects of different doses of captopril and losartan on the SBP of WKY (A and B) and of SHR (C and D). Note that captopril and losartan significantly and dose-dependently restitute the SBP in the SHR, whereas there was no significant effect in the WKY (∗, p < 0.05; one-way ANOVA, n = 3).](image-url)
either CV or IAS hypertension in SHR. This is in agreement with previous reports showing the absence of adverse effects of captopril and losartan in normotensive rats and humans (Lee et al., 1991; Horita et al., 2004; Cosentino et al., 2005).

Present data support the previous concept that the basal tone in the IAS of normal rats is partially mediated by the interaction of Ang II with the AT1-R (De Godoy et al., 2004; De Godoy and Rattan, 2005). We also observed in one of four normotensive animals that the higher dose of captopril induces a defecation reflex, resulting in the expulsion of fecal pellets (Fig. 4A). This effect was not encountered in any of the SHR (Fig. 4B) in which higher doses of captopril or losartan do not reduce the basal IASP below the normotensive levels. Such observations may provide an explanation for the lack of symptomatic anal incontinence after captopril specifically in hypertensive patients.

The above symptomatic changes in the IAS of normotensive rats were not observed with losartan. A possible mechanism for the distinct effects of captopril in the normotensive animals may be a potentiation effect by captopril on bradykinin. Accordingly, in addition to ACE inhibition, captopril is known to inhibit the degradation of bradykinin, a circulating peptide that produces smooth muscle relaxation (Engel et al., 1972). In support of this notion, we have observed that bradykinin is selectively and significantly more potent in producing IAS relaxation in WKY in comparison with SHR (data not included).

In agreement with the earlier concept of the autocrine control of RAS in the IAS (De Godoy and Rattan, 2005), the levels of Ang II in the IAS MBP were significantly higher compared with those in the RSM and AoSM in normotensive WKY (Fig. 7, B and C). These levels in the IAS increase severalfold further in SHR. The authenticity of Ang II measurements in the MBP and in the plasma (Fig. 6) was ascertained by the precipitous fall in these levels in the presence of an ACE inhibitor.

In contrast to the effect of captopril on Ang II levels in the plasma and MBP, losartan increases Ang II levels in the SHR. These findings may be explained on the bases of Ang II
displacement from the receptor binding sites by competition with the AT1-R antagonist (Zhu et al., 2004).

Higher levels of Ang II in SHR were accompanied by up-regulation of the RAS in all tissues examined. Interestingly, however, AoSM does not express RT-PCR (Fig. 10B) and Western blot products (Fig. 8B) for renin. In contrast to the clear demonstration of the RAS up-regulation at the translational level in SHR, at the transcriptional level, all tissues show lower levels of Ang gene transcripts, and no significant differences in renin and ACE (shown by RT-PCR data).

These observations suggest that up-regulation of the RAS occurs at the post-transcriptional level (Figs. 8–10). Up-regulation of the AT1-R in SHR, however, may occur at both the transcriptional and translational levels (Figs. 9B and 10D).

Previous studies from our laboratory have shown that Ang II causes direct contraction of the rat IAS via AT1-R (Fan et al., 2002; De Godoy and Rattan, 2005). In the present studies we compared the effects of different concentrations of Ang II in the basal tone of WKY and SHR IAS after treatment with captopril. Captopril was used to eliminate interference of endogenous Ang II production. The data reveal no significant difference in the potencies of Ang II between WKY and SHR (Fig. 11) despite the increase in the number of AT1-R in SHR. The maximal biological response may be achieved at agonist concentrations lower than those required to occupy all of the available receptors (Zhu, 1993). Because of this, the above findings in the IAS do not negate the physiological relevance of an increase in the number of AT1-R in SHR. Based on the theory of spare receptors (Zhu, 1993) we hypothesized that if increases in the numbers of AT1-R are of functional importance, less Ang II would be necessary to compete with losartan.
tan to produce 50% of the maximal response in SHR than in WKY. Our observations are in agreement with this concept (Fig. 11; Table 3).

In summary, the present data demonstrate hypertensive IAS in the SHR associated with the up-regulation of the RAS in the IAS. These data, along with the medical report associating CV hypertension with the hypertensive IAS, suggest a link between the two. The ability of captopril and losartan to normalize elevated IASP in the hypertensive SHR suggests the potential use of such agents in certain anorectal pathologies.

**TABLE 3**
Values of pEC\textsubscript{50} and E\textsubscript{max} for Ang II in the IAS of WKY and SHR (in the presence of 10 \textmu M captopril)

<table>
<thead>
<tr>
<th>Group</th>
<th>pEC\textsubscript{50}\textsuperscript{a}</th>
<th>E\textsubscript{max}\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>8.5 ± 0.1</td>
<td>36.6 ± 5.1</td>
</tr>
<tr>
<td>WKY (losartan 100 nM)</td>
<td>6.4 ± 0.1\textsuperscript{c}</td>
<td>28.4 ± 5.4</td>
</tr>
<tr>
<td>SHR</td>
<td>8.6 ± 0.2</td>
<td>44.3 ± 8.0</td>
</tr>
<tr>
<td>SHR (losartan 100 nM)</td>
<td>7.2 ± 0.2\textsuperscript{d,e}</td>
<td>43.1 ± 4.7</td>
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</tbody>
</table>

\textsuperscript{a} pEC\textsubscript{50} is defined as the \textsuperscript{-log EC\textsubscript{50}} (concentration of the agonist that produces 50% contraction). Data represent the means ± S.E.M. of four independent determinations.

\textsuperscript{b} E\textsubscript{max} is defined as the maximal contraction elicited by the agonist.

\textsuperscript{c} p < 0.05 compared with WKY.

\textsuperscript{d} p < 0.05 compared with SHR.

\textsuperscript{e} p < 0.05 compared with WKY (100 nM losartan).

Fig. 10. Gene transcripts of angiotensinogen (A), renin (B), angiotensin-converting enzyme (ACE) (C), and the AT\textsubscript{1}-R of IAS, RSM, and AoSM samples from WKY and SHR (D). Data are shown as the percentage relative density calculated as the IOD of the band of interest normalized by the IOD of the band of \alpha-actin (mean ± S.E.M.; n = 3).

Fig. 11. Concentration-response curves for Ang II in the internal anal sphincters isolated from WKY and SHR. These experiments were carried out in the presence of the ACE inhibitor captopril (10 \textmu M) to eliminate the effect of endogenous Ang II production. Note that the smooth muscle strips from the SHR group are more resistant to AT\textsubscript{1}-R inhibition by losartan than the WKY (+, p < 0.05; n = 4).
disorders associated with hypertensive IAS (Sun et al., 1992; Cook et al., 2001; Azpiroz and Whitehead, 2002). Based on the data in the SHR, normalization of the IAS tone to the levels of normotensive patients (without lowering it further) may provide a plausible explanation for the lack of anorectal incontinence in hypertensive patients receiving ACE and AT\(_1\)-R inhibitors.

References


Celik AF, Katsinelos P, Read NW, Khan MI, and Donnelly TC (1995) Hereditary disorders associated with hypertensive IAS (Sun et al., 1992; Cook et al., 2001; Azpiroz and Whitehead, 2002). Based on the data in the SHR, normalization of the IAS tone to the levels of normotensive patients (without lowering it further) may provide a plausible explanation for the lack of anorectal incontinence in hypertensive patients receiving ACE and AT\(_1\)-R inhibitors.

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